Methods: Cartilage samples were obtained from multi-organ donors at the University Hospital of Coimbra with approval by the Ethics Committee. After enzimatic digestion, isolated chondrocytes were cultured under non proliferating conditions to avoid dedifferentiation. Essential oils prepared by distillation of the aerial parts of *Lavandula luisieri*, *Otanthus maritimus, Laserpitium elasii, Annona muricata* and *Eryngium duriae* subsp. *juresianum* were added to the chondrocyte cultures, in at least 3 different concentrations, ranging from 0.0005 to 0.005% (v/v), 30 min before addition of IL-1, 10 ng/mL and further incubated for 18 h. The MTT reduction assay was used to rule out cytotoxic effects. NO levels were measured by a colorimetric method based on the Griess reaction. iNOS levels were evaluated by western blot.
Results: Of the essential oils tested, 2 showed a significant ability to decrease IL-1-induced NO production and iNOS protein levels. *L. luisieri* promoted the greatest inhibition (49.9±5.7%) in a concentration of 0.005% (v/v). A concentration 5x lower, 0.0025%, still promoted a significant decrease (30.0±3.1%) of NO levels. Similarly, the essential oil from *E. duriae* subsp. *juresianum* in concentrations of 0.0025% and 0.001% (v/v) decreased NO production by 38.8±12.8% and 24.6±3.7%, respectively %. Both oils significantly reduced iNOS levels. The effects observed were achieved with concentrations that did not induce cytotoxicity.
Conclusions: Among the essential oils screened, those from *L. luisieri* and *E. duriae* subsp. *juresianum* are promising samples for further fractionation in order to purify and identify the active component(s). Future studies will be designed to fully characterize the mechanism of action of such compound(s) and determine their potential usefulness as anti-osteoarthritic agents.

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EVIDENCE-BASED RECOMMENDATIONS ON DULOXETINE TREATMENT FOR THE PAIN IN KNEE AND HIP OSTEOARTHRITIS: SYSTEMATIC REVIEW AND EXPERT CONSENSUS

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Background: Pain is a major problem concerning patients with Osteoarthritis (OA). Duloxetine has shown to be effective as a central acting analgesic in chronic back pain, fibromyalgia and other rheumatic conditions
Purpose: The aim of this study was to develop evidence-based recommendations for the use of Duloxetine in the pain of patients with OA of hip or knee.
Methods: A scientific committee from the Mexican College of Rheumatology (CMR) selected clinically relevant questions concerning the use of Duloxetine in OA. A systematic review of the literature for randomized controlled trials (RCT) with Duloxetine as treatment for the pain management in patients with OA was performed blinded by two experts in systematic review.
Research strategy: We searched RCT using Duloxetine OA in Medline/PubMed, EMBASE, ARTEMISA, LILACS and the Cochrane Library in English or Spanish languages.
Selection Criteria: We included RCT, in which adults with knee and or hip OA were treated with Duloxetine, considering as efficacy outcome variables: pain and or functional capacity, and safety: adverse events. Quality of the articles was evaluated by ten experts with the Jadad Score and CONSORT guidelines 2010. The level of evidence was appraised according to the Oxford Levels of Evidence. An expert consensus meeting took place in April 2011 including 12 rheumatologists when the scores of Jadad and Consort evaluations were revised. Recommendations were made during interactive workshops where the evidence was reviewed. Agreement among participants and the impact of the recommendations were systematically assessed using voting procedure in a plenary session.
Results: A total of 12 references were identified, among which nine were excluded because the intervention evaluated other conditions and or consider animal models; two articles were included for efficacy and one for safety. There was a good quality of evidence in the three articles assessed, in two rounds of discussion between experts. The recommendations were formulated, including usefulness, population, optimal dosage and safety. For efficacy Duloxetine was evaluated by experts as useful considering those patients with persistence intensive pain (visual analogue scale >4), and with previous use of other analgesics, without correlation with the disease stage (clinical and radiologic) The secondary adverse events where reported correctly in the RCT and a safe profile was identified by consensus, looking more events with high dose, but resolved with a scaling dosage administration: starting at 30 mg the first week, and then increase at 60 mg
Conclusions: Duloxetine is an effective and safety option for the management of pain in OA of hip and knee in patients with history of analgesics or NSAIDS and persistency of pain (more than 4 in VAS), independently of the radiologic stage, at a starting dose of 30 mg for one week an after 60 mgd, ingested with food. These recommendations are evidence based and supported by a panel of the Mexican College of Rheumatology specialists and experts. Further research has to be place to evaluate the real cost-effectiveness of Duloxetine in the management of pain in OA patients.

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A COMPARISON OF ANTI-INFLAMMATORY PROPERTIES OF WHOLE BLOOD, PLATELET-RICH PLASMA, AND AN AUTOLOGOUS PROTEIN SOLUTION IN IL-1β- AND TNFα-STIMULATED CHONDROCYTES

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Purpose: Autologous intra-articular injections of platelet-rich plasma (PRP) have been investigated as a potential treatment for cartilage degeneration. Degradation of the cartilage matrix, seen in early stage osteoarthritis (OA), is believed to be driven by inflammatory cytokines such as IL-1 and TNFα. These cytokines induce cells in the joint to produce matrix metalloproteinases (MMPs) that in turn are responsible for degradation of the cartilage matrix. An autologous protein solution (APS), which is derived from PRP and contains anti-inflammatory cytokines, has been developed. The purpose of this study was to determine if APS can inhibit the production of MMP-13 from IL-1β- and TNFα-stimulated chondrocytes more effectively than PRP.
Methods: *Preparation of samples*: Nine ml of PRP was prepared from 90 ml fresh whole blood (8.3% v/v ACD-A) using two disposable separation devices containing a tuned density buoy (Biomet Biologics). Six ml of the combined PRP output was transferred to a disposable device containing polyacrylamide beads. The device was centrifuged and the APS was collected. Cells in the whole blood (WB), remaining PRP, and APS were lysed by processing 500 µl of each sample at 5°C with protease inhibitor and cell disruption glass beads in a cell disrupter (Scientific Industries). The samples were centrifuged at 5°C for 20 minutes at 13,000 rpm (11,500 g), and the lysate was removed and stored (−50°C) until use in the assay.
Cell Assay: Human knee articular chondrocytes (NHAC, P5, Lonza Inc.) were seeded in 12-well plates at 20,000 cells/cm^2 in 2 ml growth media (Lonza). Two hours prior to the assay, media was exchanged with serum-free. The treatment wells were pre-incubated with 25 µl WB, PRP, or APS in a transwell insert (Corning Inc.) for 2 hours before addition of recombinant IL-1β (5 ng/ml) (Sigma) and TNFα (100 ng/ml) (Prospec). Negative control was untreated media, and positive control contained only IL-1β (5 ng/ml) and TNFα (100 ng/ml). Following incubation at 37°C and 5% CO_2 for 24 hours, the supernatant was removed and frozen at −50°C. Cells were trypsinized and counted. The supernatant was assayed for MMP-13 by ELISA (R&D Systems), and MMP-13 production was normalized to cell number.
Statistical Analysis: A single-factor ANOVA with a post-hoc Fisher LSD test (α = 0.05) was performed to determine statistically significant differences.
Results: APS was more capable of inhibiting MMP-13 production by IL-1β- and TNFα-stimulated chondrocytes than an equal dose of WB ($p < 0.0001$) or PRP ($p = 0.0003$). WB had no effect on MMP-13 production compared to the positive control ($p = 0.4$). PRP and APS inhibited approximately 45% and 70% of MMP-13 production, respectively.